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Spindle checkpoint silencing: ensuring rapid and concerted anaphase onset

Kevin G Hardwick^{1*} and Jagesh V Shah²

Addresses: ¹Wellcome Trust Centre for Cell Biology, University of Edinburgh, King's Buildings, Mayfield Road, Edinburgh, EH9 3JR, UK;

²Department of Systems Biology, Harvard Medical School, 4 Blackfan Circle, HIM 564, Boston, MA 02115, USA

* Corresponding author: Kevin G Hardwick (kevin.hardwick@ed.ac.uk)

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Abstract

The spindle checkpoint delays anaphase onset in the presence of defective kinetochore-microtubule attachments. Such delays can last for just a few minutes or several hours, but very shortly after all chromosomes achieve bi-orientation, a remarkably synchronous anaphase ensues. We are beginning to understand the pathways involved in silencing spindle checkpoint signals and subsequent activation of the anaphase-promoting complex. Here, we review recent advances made in our understanding of the molecular mechanisms regulating this critical cell cycle transition.

Introduction and context

Mis-segregation of chromosomes in the germline leads to aneuploidy and spontaneous abortion or birth defects and, in the soma, is associated with several diseases, including cancer [1]. Despite the complexities of mitosis, such segregation defects are in fact extremely rare. The high fidelity of this process is due, in large part, to the action of checkpoints (surveillance systems) that coordinate the successful completion of cell biological processes (DNA replication and spindle assembly) with cell cycle progression. During mitosis, the spindle checkpoint monitors kinetochore-microtubule interactions and delays anaphase onset until all sister-chromatid pairs are attached and bi-oriented on the mitotic spindle [2]. Its components, such as the Mad (mitotic arrest defective) and Bub (budding uninhibited by benomyl) proteins, do this by inhibiting Cdc20, which is an activator of the mitotic E3 ubiquitin ligase known as the anaphase-promoting complex or cyclosome (APC/C). By inhibiting the APC/C, securin and cyclin B are stabilized, thereby maintaining sister-chromatid cohesion and high levels of mitotic CDK (cyclin-dependent kinase) activity [3,4].

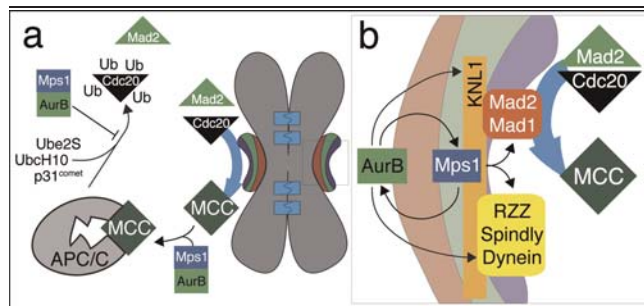
Checkpoint signals (e.g., Mad2-Cdc20 and mitotic checkpoint complexes, or MCCs) are generated at

unattached kinetochores and then must be amplified throughout the cytoplasm to ensure global inhibition of the APC/C (Figure 1). Kinetochore often bind laterally to microtubules at first, but then these microtubule interactions will mature to form the more appropriate stable, end-on, bipolar attachments that 'satisfy' the spindle checkpoint. These attachments are under tension and it is thought that this spatially separates and constrains kinase and phosphatase activities within distinct centromeric and kinetochore regions: such subtleties may be key to determining the overall balance of checkpoint signal production versus silencing.

Once such attachments are achieved, several kinetochore-based factors act coordinately to stop kinetochore-mediated Mad2-Cdc20 complex generation (see below and Figure 2). However, preventing Mad2-Cdc20 production at kinetochores is not sufficient and the existing inhibitors in the cytoplasm and any cytoplasmic amplification mechanisms must be rapidly quenched to ensure a timely and synchronous anaphase [4,5].

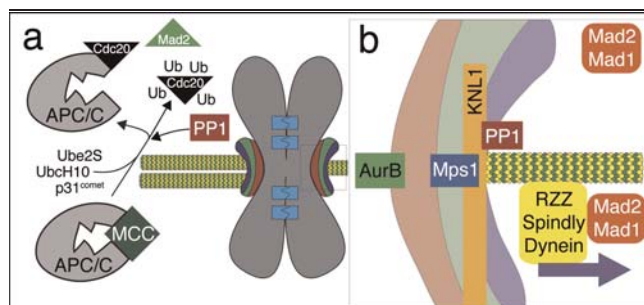
Do kinetochore and cytoplasmic silencing mechanisms act cooperatively or in distinct pathways? Here, it is important to note that some model systems may be

Figure 1. APC/C inhibition is regulated by signalling within the cytoplasm (a) and at the kinetochore (b)



(a) The inhibition of the anaphase-promoting complex/cyclosome (APC/C) occurs primarily in the cytoplasm through the generation and amplification of kinetochore-generated signals that are balanced by the dissociation of inhibitory complexes. Checkpoint kinases (e.g., Mps1 and AurB) are thought to act on this dissociation pathway to stabilize the inhibited form of the APC/C. **(b)** These same kinases act at the kinetochore to activate and maintain the checkpoint through the regulation of the local generation of the wait signal. This activity keeps checkpoint generators such as the Mad1/Mad2 and the Spindly/RZZ/dynein complexes localized to kinetochores through the action of kinases on scaffolds such as kinetochore-null 1 (KNL1) and checkpoint activators such as dynein. AurB, Aurora B kinase; Mad, mitotic arrest defective; MCC, mitotic checkpoint complex; Mps1, monopolar spindle 1; RZZ, rough deal, zeste white 10, zwilch.

Figure 2. Silencing of the checkpoint acts both in the cytoplasm (a) and locally at each kinetochore (b) upon microtubule attachment



At a single kinetochore, the binding of the microtubule and subsequent recruitment and activation of protein phosphatase 1 (PP1) locally reduce checkpoint kinase activity and release checkpoint complexes via dissociation of Mad1/Mad2 or translocation along microtubules via dynein. Loss of the single generators culminates in the total loss of generation when all chromosomes become attached. The remaining cytoplasmic inhibitory complexes (MCC-APC/C) are dissociated in part by the natural decay in the absence of generation and also by the activation of pathways that enhance dissociation to produce the synchronous onset of anaphase. The dissociation activity results in the activation of the APC/C by Cdc20, the ubiquitination and degradation of cyclin B and securin, and the onset of anaphase. APC/C, anaphase-promoting complex/cyclosome; AurB, Aurora B kinase; KNL1, kinetochore-null 1; Mad, mitotic arrest defective; MCC, mitotic checkpoint complex; Mps1, monopolar spindle 1; RZZ, rough deal, zeste white 10, zwilch.

more reliant on cytoplasmic (or nucleoplasmic in the closed mitosis of yeast) versus kinetochore-based generation of inhibitory signals. How does silencing differ in these varied systems? In this report, we outline some of the recent advances in our mechanistic understanding of spindle checkpoint silencing.

Major recent advances

Major recent advances have been made in areas of kinase (Aurora and monopolar spindle 1 [Mps1]) and phosphatase (protein phosphatase 1 [PP1]) signaling, dynein-mediated stripping of checkpoint proteins from kinetochores, and proteolytic turnover of checkpoint components. Other silencing pathways (such as p31^{comet}) exist, but little has been learned of late with regard to their modes of action.

Balancing kinases/phosphatase activity

Aurora B and protein phosphatase 1

In fission yeast, Aurora kinase 1 (Ark1) activity is required to maintain spindle checkpoint arrest, and when its kinase activity is inhibited (using *ark1-as*, an ATP analog-sensitive allele), kinetochore localized PP1 activity (Dis2 in *Schizosaccharomyces pombe*) is also required to silence the checkpoint and reactivate the APC/C [5]. Budding yeast also uses a PP1 homolog (Glc7 in *Saccharomyces cerevisiae*) in silencing the spindle checkpoint [6] in concert with a localization factor, Fin1 [7]. Most recently, it was demonstrated in vertebrates that the gamma isoform of PP1 is recruited via KNL1 (kinetochore-null 1) binding to kinetochores, where it opposes Aurora B activity to stabilize microtubule attachments [8]. This creates a bi-stability, with either Aurora B or PP1 activity dominating in the outer kinetochore, thereby revealing an important switch to a stabilized kinetochore-microtubule attachment that may initiate kinetochore-mediated spindle checkpoint silencing. Ndc80, MCAK (mitotic centromere-associated kinesin), and KNL1 are all Aurora and PP1 substrates that influence microtubule stabilization, but the substrates that are most important for checkpoint silencing remain to be identified, although KNL1, Ndc80, and dynein are candidates [8-11].

Monopolar spindle 1 kinase (and opposing PPases)

Mps1 inhibition is a powerful suppressor of the checkpoint in mammalian cells. RNA interference (RNAi) of Mps1 shortens mitosis [12-14] much like the depletion of Mad2 and BubR1 first demonstrated by Meraldi, Draviam, and Sorger [15]. Mps1 inhibition within mitosis, through the use of small-molecule inhibitors, is equally potent [16-18]. This supports models in which Mps1 kinase carries out functions in 'wait' signal generation at the kinetochore, such as promoting localization of Mad1/Mad2, and also in

'wait' signal stabilization in the cytoplasm via a mechanism that has yet to be described.

Given the complex phenotypes associated with the loss of these checkpoint kinases, we propose that Aurora and Mps1 kinases are tri-functional (Figure 1) and can (a) regulate microtubule attachments, (b) regulate 'wait' signals at the kinetochore, and (c) stabilize 'wait' signals in the cytoplasm. As chromosomes attach, the reduced local activity of these kinases results in kinetochore composition changes that stabilize the microtubule attachment and reduce wait signal generation (Figure 2).

Loss of kinetochore-localized spindle checkpoint components

Dynein/Spindly/RZZ

Spindly and the RZZ (rough deal, zeste white 10, zwilch) complex are required for dynein recruitment to kinetochores. Upon microtubule attachment, several outer kinetochore components, including Mad1-Mad2, are transported to spindle poles in a dynein-dependent fashion [19,20]. This motor-mediated removal of the Mad1-Mad2 scaffold has been proposed to eliminate wait signal generation by splitting the essential catalytic platform into an inactive form [21]. It was recently shown that dynein-dependent removal of Spindly from microtubule-attached kinetochores is required for checkpoint silencing. Spindly 'motif' mutants that cannot bind dynein prevent dynein recruitment to kinetochores and result in persistent checkpoint signaling with persistent localization of the Mad1/Mad2 to the attached kinetochore [22]. However, in the complete absence of Spindly, after RNAi-mediated depletion, there exists a mechanism to delocalize Mad1/Mad2 complexes from attached kinetochores, perhaps revealing a conserved silencing pathway present in lower eukaryotes without obvious Spindly/RZZ homologs.

The removal of the wait signal generator from the attached kinetochore occurs through at least two pathways: dissociation and physical translocation along the attaching microtubules themselves. This disruption of signal generation occurs at each chromosome. After the total number of unattached chromosomes is reduced to zero, the loss of the signal generation is followed by the release of inhibition that is already acting on the APC/C.

Relieving inhibition on the anaphase-promoting complex/cyclosome

Ubiquitination activity

During mitosis, unattached kinetochores generate inhibitory complexes (e.g., MCC) that result in the significant accumulation of inhibitory APC/C complexes (MCC-APC/C) [23-25]. Dissociation of these complexes is required to permit APC/C activation. Work by Reddy

and colleagues [26] identified a role for ubiquitination in the dissociation of this complex via the direct ubiquitination of Cdc20. Their work established that the E2 enzyme UbcH10, in concert with p31^{comet}, could act to ubiquitinate Cdc20 in the context of the MCC-APC/C complex, providing the first direct mechanism for relief of APC/C inhibition. However, Cdc20 turnover is also critical for checkpoint arrest [27-29], and a full understanding of the complex regulation of Cdc20 remains to be realized.

More recently, another E2 ubiquitin enzyme, Ube2S, has been shown to be critical for rapid anaphase onset after release from extended drug-induced checkpoint activation. Surprisingly, loss of the enzyme during a normal, unperturbed mitosis has, at best, a modest delay on anaphase onset, indicating a specific role during extended checkpoint activation. Perhaps more importantly, the loss of Ube2S in an extended checkpoint arrest reveals a decoupling between kinetochore-mediated silencing and cytoplasmic APC/C activation [30-32].

A key feature of a synchronous anaphase onset is the coupling of MCC-APC/C complex dissociation (i.e., APC/C activation) to kinetochore attachment, which is proposed here to occur via inhibition of the checkpoint kinases Mps1 and Aurora B kinase (AurB) and activation of PP1. This coupling would speed anaphase onset after final kinetochore attachment, a commonly made and enigmatic observation [4].

Future directions

Whereas mechanistic details of silencing pathways are being clarified on a monthly basis, the overall control and any coordination of these pathways are far from clear. Inter-module coordination could occur via AurB-mediated recruitment of PP1 to KNL1. These activities can feed into regulation of Spindly-dynein transport as kinetochore targeting of dynein is under PP1 regulation [11]. However, non-stripping pathways of Mad1-Mad2 release must also be regulated, and identification of other AurB/Mps1/PP1 targets both at kinetochores and in the cytoplasm will be required. Finally, the wealth of quantitative timelapse and biochemical measurements provide a robust substrate for computational modeling that will have a major role in testing the many molecular hypotheses.

Abbreviations

APC/C, anaphase-promoting complex/cyclosome; Ark1, Aurora kinase 1; AurB, Aurora B kinase; Bub, budding uninhibited by benomyl; KNL1, kinetochore-null 1; Mad, mitotic arrest defective; MCC, mitotic checkpoint complex; Mps1, monopolar spindle 1; PP1, protein

phosphatase 1; RNAi, RNA interference; RZZ, rough deal, zeste white 10, zwilch.

Competing interests

The authors declare that they have no competing interests.

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